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Detection of antibiotic residues in poultry carcasses in Mashhad poultry abattoir

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Abstract

In poultry breeding, use of any antibiotics as a food additive that promotes growth, prevention or treatment of infectious diseases will increase the chance of drug residues in poultry meat products.

In this study, the presence of different antibiotics in ten broiler flocks, which were later transported to Mashhad poultry abattoir have been investigated, using four plate test (FPT) method. A total of one hundred breast muscle and sixty liver samples (10 breast muscles and 6 liver samples from each flock) were collected randomly.

The results of this study showed, the 18.75% of samples were positive for antibiotic residues and 6.88% of samples were suspected. The results also showed that sulfonamides residue in meat and liver samples were higher than other investigated antibiotics (22% in meat and 11.7% in livers). It can be concluded that the withdrawal time of antibiotics before slaughter has been ignored in many of these farms therefore, the administration of antibiotics in poultry farms must be more rigidly controlled to prevent drug residues in food-producing animals.

Keywords: Antibiotic residues, poultry, meat, liver, withdrawal time, four plate test

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Introduction

Chicken meat provides not only high-quality protein, but also important vitamins and minerals (Charlton *et al*., 2008). Besides, chicken meat has high level of essential polyunsaturated fatty acids, like omega (n)-3, which can be easily digested and efficiently absorbed (Cerina *et al*., 2011). In addition to nutritional value, chicken is more popular due to the fact that it is relatively easy to prepare and is more affordable than other types of meat. In the past few decades, antibiotics have been extensively used in livestock animals as food additive for growth promotion, prevention or treatment of infectious diseases.

According to the FDA, about 87% of antibiotics used in livestock animals are for treatment, control or prevention of infectious diseases and 13% as food supplement for nutritional purposes to increase growth and productivity (Meredith et al., 1965; Dipeolu and Alonge, 2002; Donoghue, 2003; Mahgoub *et al*., 2006). Due to the extensive non-therapeutic use of antibiotics and lack of adequate control of administration, the risk of drug accumulation and their residuals in animal tissues and their product will increase (Meredith *et al*., 1965; Lemus, 2008).

Antibiotic residues and their metabolites in poultry meat may cause several adverse effects on consumers. They can cause direct toxicity, developing resistant bacteria, and allergies, even when used at very low doses (Kirbis *et al*., 2007).

In addition to direct toxicity, normal micro flora in digestive system can adapt and acquire resistance to the antibiotic by long term overexposure to trace amounts of antibiotics through contaminated foods (Javadi *et al*., 2011; Myllyniemi *et al*., 2004).

Concerns about the risk of antibiotic residues resulted in establishing the maximum limits of drug residues (MRLs), which are the maximum amount of residues that could legally permitted to be in the food product without causing adverse effects to the consumers (Myllyniemi *et al*., 2004; Reyes-Herrera., 2005).

Several methods have been developed for determining the residues of antibiotics in poultry tissues, including microbiological methods, chromatography methods, ELISA and immunochemical methods (Kaya and Filazi., 2010). The microbiological methods are the methods of choice since they are cheap, easy to perform and able to measure a large number of samples (Karraouan *et al*., 2009; Kirbis *et al*., 2007; Javadi *et al*., 2011). Due to the high sensitivity and specificity of the Four Plate Test (FPT), it has been used by several researchers and also has been accepted by the European Union as a standard method for determining antibiotic residues. The aim of this study was to investigate the possible presence of main groups of antibiotic in broiler carcasses, including sulfonamides, aminoglycosides, microlides, tetracyclines and β-lactam family. It should be noted that measuring the level of antibiotic residues was not the purpose of this study.

Materials and methods

Meat samples: A total of 100 breast muscle samples and 60 liver tissue samples were collected randomly from ten different broiler flocks which were transferred to Mashhad poultry abattoir (10 breast muscles and 6 liver samples from each flock). Samples were labeled and placed on ice in suitable transport boxes and transferred to laboratory and kept in -20°C before being tested.

The lyophilized bacteria including *Bacillus subtilis* and *Micrococcus luteus* were kindly donated by Khorasan Razavi veterinary administration. The bacterial suspension were prepared from its lyophilized form and then cultured in nutrient agar media.

The agar plates were incubated at 37°C for 24 hours. Bacterial suspensions equivalent to 0.5 McFarland turbidity were prepared from typical colonies of *Bacillus subtilis* and *Micrococcus luteus*, using sterile saline solution. Under aseptic conditions, the bacterial suspensions were cultured on plates containing

agar media, using sterile cotton swabs. Agar media for *Bacillus subtilis* were *prepared in three* different *pH* levels (6, 7.2 and 8) and for *Micrococcus luteus* were adjusted to pH 8. The pH of all media was adjusted with normal solution of HCl and NaOH using a digital pH meter. The plates for *Bacillus subtilis* were incubated at 30°C and for *Micrococcus luteus* at 37°C for 24 hours.

In this study, three different media were made with three different pH. For pH=6 the culture medium consisted of 3.45g/L tryptic casein peptones (Que lab, Canada), 3.45g/L tryptic meat peptones, 5.1 g/L sodium chloride and 13g/L agar agar. For pH=7.2 the culture medium consisted of 7g/L tryptic meat peptones (Que lab, Canada), 5 g/L sodium chloride, 0.8 g/L sodium triphosphate and 13g/L agar agar. For pH=8 the culture medium consisted of 3.45g/L tryptic casein peptones, 3.45g/L tryptic meat peptones (Que lab, Canada), 5.1g sodium chloride, and 13g/L agar agar.The meat and liver samples were cut into cylinders to form disks (8mm diameter, 2mm thick and weighing approximately 0.5g) by use of a surgical punch and individually placed on each labeled cultured plates using sterile forceps (at a distance of 1 cm from the edges). All samples were evaluated after 24 hours.

The samples with 2mm inhibition zones or more were considered as positive to indicate the presence of antibiotic residues while the sample with 1-2 mm inhibition zones were considered as suspected and the sample with less than 1 mm inhibition zones were considered as negative.

Results

Based on the diameter of the inhibition zone (2 mm or more), 30 samples (18.75%) were determined to be positive and 11 samples (6.88%) were suspected while the rest of samples (74.37%) with less than 1 mm of inhibition zone were declared as negative (Fig.1). In order to compare the presence of antibiotic residues in different flocks, Fischer exact test was used. There was a significant difference between flocks in antibiotic residues of their examined tissues (p=0.022 and P<0.001).

According to our results, 8 (13.3%) of liver samples and 22 (22%) of meat samples were positive at least for one group of antibiotic residues and there was no difference between liver and meat samples (p =0.174).

Residues of β-lactam and tetracycline were not detected in any of liver and meat samples, while the sulfonamide were detected in 7 (11.7%) of liver samples and 22 (22%) of meat samples however, this difference was not significant (P=0.1). Aminoglycosides were detected in 3 (5%) of liver samples and in 1 (1%) of meat samples, this difference also was not significant (p =0.119). Macrolides were detected in 4 (6.7%) of liver samples and in none of meat samples (0.0%), this difference was significant (p =0.019) (Fig 1).

Discussion

Chicken meat has high quality proteins and low fat, along with important vitamins and minerals (Givens, 2005). Also chicken production is quicker and cheaper than other meat sources (Ivanovic, 2003). By forbidding of some animal meats consumption, like pork, due to religious rules in Islamic countries, and also higher price of red meats like beef and lambs etc., chicken meat gains more attention in these countries and plays an important role in public nutrition. An increased demand for chicken meat, forces the poultry breeding industry to produce more amount of meat, which then leads to a further increase in the use of drugs such as antibiotics or hormones for growth stimulation and weight promotion (Paryad *et al*., 2008).

Due to the high risk of veterinary drug residues in foods of animal origin, the maximum residues limit (MRL) regulation for use of each pharmacologically active substance has been developed by European Union (EU). The MRL regulation determines the maximum concentrations of residues which are permitted in foods of animal origin and should be followed for public health food safety (Myllyniemi., 2004; Reyes-Herrera *et al*., 2005)

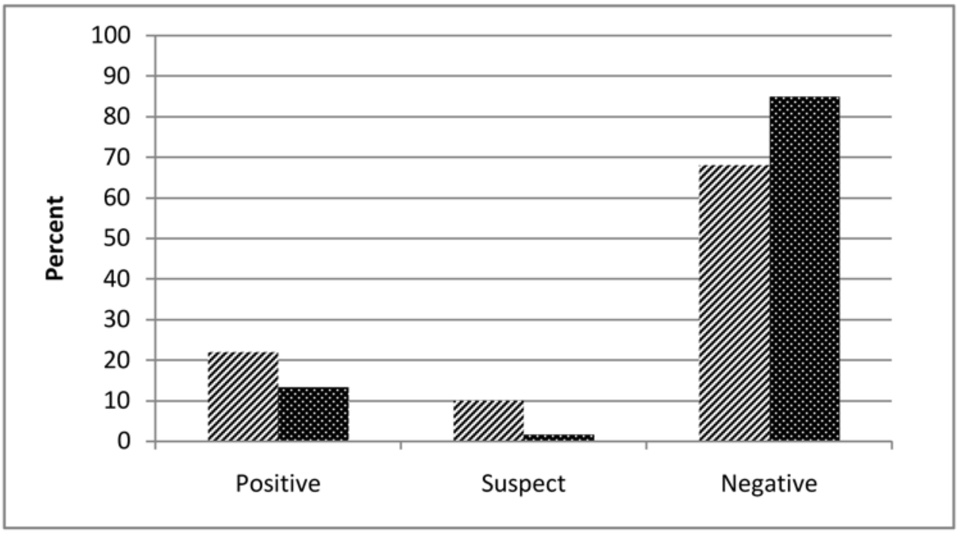


Figure 1. Comparison of antibiotic residues in breast (gray) and liver tissue samples (black). Percentage of positive, suspicious and negative sample results.

The occurrence of antibiotic and other veterinary drug residues in poultry products have been reported around the world (Amuda-Giwa., 1994; Oboegbulem and Fidelis., 1996; A-Ghamdi *et al*., 2000; Kabir *et al*., 2004., etc.).

For monitoring of antibiotic residues, microbiological methods are easy, rapid, effective and simple tests to perform (Stead *et al*., 2004). However, this method cannot detect all known antibiotic residues in foods of animal origin (Korsrud *et al*., 1998). In addition to detecting antibiotic residues in chicken meat, this method has been applied to monitor antibiotic residues in other foods such as eggs and red meats (Saitanu and Amornsin., 1994; Hussein *et al* 2005; Lynas *et al*., 1998). For example, Saitanu and his colleagues by use of this method showed the presence of antibiotic residues in 8.4 % of 1461 shrimp samples (Saitanu and Amornsin., 1994).

In Iran, a little work has been done to demonstrate the presence of antibiotic and pesticide residue in poultry products.

In 2009, Javadi and colleagues by use of FPT method, reported antibiotic residue in 62.5% (25 out of 40 samples) of broiler meat and in 100% of liver tissue samples of Tabriz poultry abattoir (Javadi *et al*., 2009). However, they did not mention the percentage of each antibiotic residue. In contrast, the results of this study showed that 18.7% of all samples have antibiotic residues. In agreement to our results, in 2010, Tajik *et al*., by use of FPT method, reported that 17.5% of liver, kidney and muscle samples of chicken carcasses as positive in three provinces in north-west of Iran (n = 160) (Tajik *et al*., 2010). However they did not report the percentage of each antibiotic residue.

The results of this study also, showed that presence of sulfonamides in chicken meat was higher than other antibiotics residues and macrolied residues were present only in the liver samples of one poultry flock. Moreover, residues of aminoglycoside antibiotics were present in 3.75% of samples.

It can be concluded that some of poultry meat providers in Mashhad do not manage the withdrawal time (the length of time that is required to metabolize and eliminate the administrated drugs). Therefore, it can be suggested that proper management strategies for controlling the veterinary drug usage in poultry farms and also monitoring the withdrawal time for antibiotics and screening the MRL in their products including livers, eggs and meats is necessary.

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جستجوی باقیماندههای آنتیبیوتیکی در لاشههای طیور در کشتارگاه صنعتی مشهد

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چکیده

در مزارع پرورش طیور استفاده گسترده از هر گونه آنتیبیوتیک که به منظور مشوق رشد، پیشگیری و یا درمان بیماریهای عفونی انجام میگیرد احتمال وجود بقایای دارویی در تولیدات گوشتی را افزایش میدهد. در این مطالعه بقایای مختلف آنتیبیوتیک در ده گله گوشتی ارجاعی به کشتارگاه طیور مشهد با استفاده از روش "آزمون چهار پلیت" مورد تحقیق قرار گرفت. در مجموع ۱۶۰ نمونه عضله سینه و ۶۰ نمونه کبد (ده عضله سینه و شش نمونه کبد از هر گله) بطور تصادفی جمعآوری شد.

نتایج این مطالعه نشان داد که ۱۸/۷۵٪ نمونهها از نظرباقیمانده آنتیبیوتیکی مثبت و۶/۸۸٪ نمونهها مشکوک به حضور باقیماندههای آنتیبیوتیکی بودند. نتایج همچنین نشان داد که باقیمانده سولفونامیدها در نمونه های کبد و گوشت بیش از سایر آنتیبیوتیکها (۲۲٪ در عضله سینه و۱۱/۷٪ درکبد) بود. از این مطالعه می توان چنین نتیجه گرفت که زمان پرهیز از مصرف آنتیبیوتیک ها در این مزارع پرورش طیور رعایت نشده است. بنابراین، استفاده از آنتیبیوتیک ها در مزارع پرورش طیوربایستی بیشترتحت نظارت قرار گیرد تا مانع از بروز بقایای آنتیبیوتیکی در فرآوردههای غذایی با منشأ دامی گر دد .

واژگان کلیدی: باقیمانده دارویی، طیور، گوشت، کبد، زمان پرهیز از مصرف دارو، آزمون چهار پلیت